American Journal of Botany 102(1): 36–49, 2015.

ECOLOGICAL NICHE DIFFERENTIATION OF POLYPLOIDIZATION IS NOT SUPPORTED BY ENVIRONMENTAL DIFFERENCES AMONG SPECIES IN A COSMOPOLITAN GRASS GENUS[1]

VERNON VISSER[2,4] AND JANE MOLOFSKY[3]

[2]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Matieland 7602, South Africa; and
[3]Department of Plant Biology, University of Vermont, Burlington, Vermont 05405 USA

- *Premise of the study:* Polyploidization frequently results in the creation of new plant species, the establishment of which is thought to often be facilitated by ecological niche differentiation from the diploid species. We tested this hypothesis using the cosmopolitan grass genus *Phalaris* (Poaceae), consisting of 19 species that range from diploid to tetraploid to hexaploid. Specifically, we tested whether (1) polyploids occupy more extreme environments and/or (2) have broader niche breadths and/or (3) whether the polyploid species' distributions indicate a niche shift from diploid species.
- *Methods:* We employed a bootstrapping approach using distribution data for each species and eight environmental variables to investigate differences between species in the means, extremes, and breadths of each environmental variable. We used a kernel smoothing technique to quantify niche overlap between species.
- *Key results:* Although we found some support for the three hypotheses for a few diploid–polyploid pairs and for specific environmental variables, none of these hypotheses were generally supported.
- *Conclusions:* Our results suggest that these commonly held hypotheses about the effects of polyploidization on ecological distributions are not universally applicable. Correlative biogeographic studies like ours provide a necessary first step for suggesting specific hypotheses that require experimental verification. A combination of genetic, physiological, and ecological studies will be required to achieve a better understanding of the role of polyploidization in niche evolution.

Key words: canary grass; ecology; niche; *Phalaris*; Poaceae; polyploidy; whole genome duplication.

Polyploidy, or whole-genome duplication (WGD), is an important mechanism of speciation in plants (Levin, 2002; Soltis et al., 2004; Wood et al., 2009; Jiao et al., 2011). It arises through either genome doubling within a single species (autopolyploidy) or hybridization of two different species in which the resulting daughter species possesses a full set of chromosomes from each parent (allopolyploidy) (Stebbins, 1947; Grant, 1975; Lewis, 1980). Polyploidy is hypothesized to have both immediate and long-term consequences on plant genetics, transcriptomics, genomics, and epigenetics (Stebbins, 1950; Lynch and Force, 2000; Soltis and Soltis, 2000; Lind-Halldén et al., 2002; Osborn et al., 2003; Liu and Adams, 2007; Jackson and Chen, 2010; Madlung, 2013; Madlung and Wendel, 2013). Hypothetically, to be successful, polyploids must occupy distinct niches from their diploid progenitors (Levin, 1975). The idea here is that a polyploid species must differentiate from the parental progenitors or will likely be outcompeted by the parental lines (Levin, 1975). Moreover, a newly formed tetraploid species may face extinction by mating with its parental diploid and creating triploid individuals that are not viable or less fertile (known as the minority cytotype disadvantage); occupying different habitats will minimize the likelihood of producing triploid offspring (Levin, 1975; Fowler and Levin, 1984; Theodoridis et al., 2013). Overall, polyploids have been hypothesized to (1) occupy more extreme environments (Hagerup, 1932; Grant, 1981) and/or (2) have overall broader niches (Stebbins, 1950; Levin, 2000; te Beest et al., 2012), and/or (3) have a clear niche shift from their diploid progenitors (Levin, 1975).

Previous ecological studies have primarily tested the hypothesis that polyploids occupy distinct niches from their diploid progenitors (e.g., Mandáková and Münzbergová, 2006; Sampoux and Huyghe, 2009; Glennon et al., 2012; Laport et al., 2012; Manzaneda et al., 2012; Godsoe et al., 2013), although some have investigated the hypothesis that polyploids occupy harsher environments and/or have broader niches than their diploid progenitors (e.g., Martin and Husband, 2009; McIntyre, 2012; Theodoridis et al., 2013). To date, only one study has investigated all three hypotheses simultaneously (Martin and Husband, 2009). Most studies have focused on a single species with different cytoptypes (but see Martin and Husband, 2009; Glennon et al., 2014 for exceptions). The few studies that have investigated multiple species have only examined a few environmental variables.

In this study, we used a cytobiogeographical approach to investigate the three main hypotheses about polyploidization and ecological niches using the canary grasses (*Phalaris* L.; Poaceae). This genus includes 19 species in four different lineages, three of which contain polyploid species (Fig. 1A; Appendix S1, see Supplemental Data with online version of this article), and whose distributions have been studied (Voshell et al., 2011; Voshell and Hilu, 2014), making them an ideal group to

[1]Manuscript received 3 October 2014; revision accepted 5 November 2014.

The authors thank Khidir Hilu and Stephanie Voshell for the *Phalaris* phylogeny and Dave Richardson for valuable comments on the original manuscript. V.V. was supported by postdoctoral grants from the Working for Water Programme via the Centre for Invasion Biology, the NRF (Scarce Skills Fellowship) and the SANBI Invasive Species Programme. J.M. acknowledges funding from NIFA/USDA HATCH and the Vermont Agricultural Experimental Station.

[4]Author for correspondence (e-mail: vervis@gmail.com)

doi:10.3732/ajb.1400432

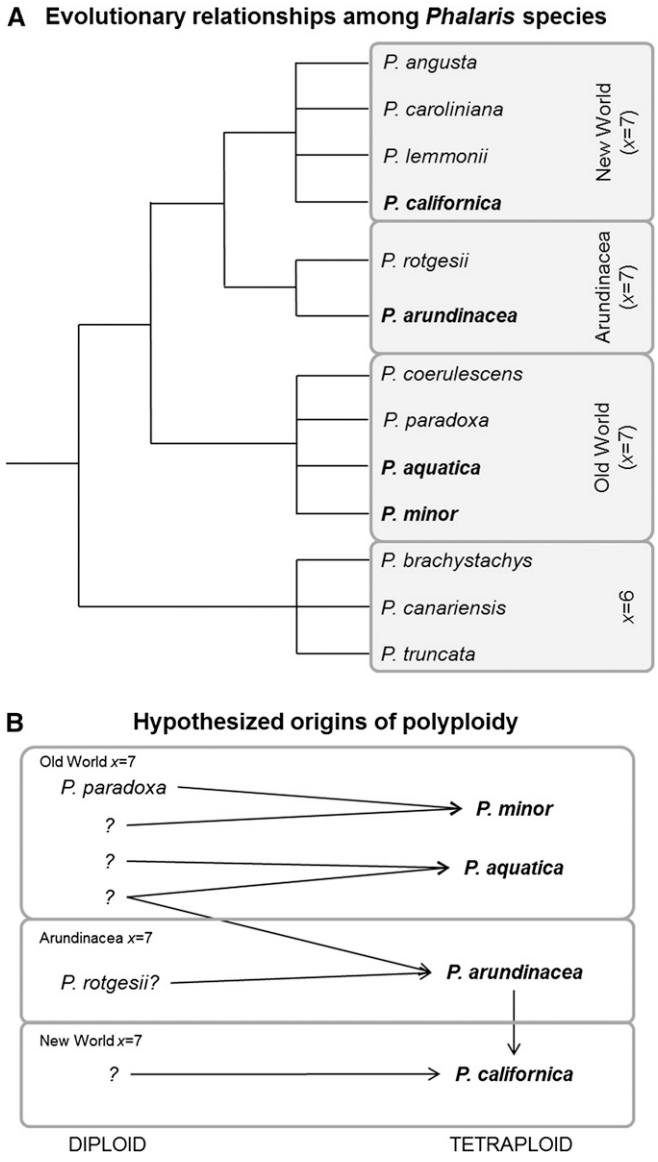

Fig. 1. (A) Evolutionary history of species within the genus *Phalaris*, adapted from Voshell and Hilu (2014) and based on their phylogeny generated using ITS sequence data. We have simplified all of the terminal node relationships to be polytomies because of conflicting placements of some species. Tetraploid species are shown in boldface. (B) Hypothesized origins of polyploid species. All tetraploids are thought to be the result of hybridization between two species (allotetraploids): Voshell et al. (2011) proposed that *P. minor* received its paternal genome from *P. paradoxa*; *P. aquatica* is thought to be the result of hybridization between two extinct diploid *Phalaris* species (Jakubowski et al., 2013). *P. arundinacea* and *P. aquatica* are thought to be quite closely related and possibly received one copy of their genomes from the same diploid ancestor (probably now extinct; Jakubowski et al., 2013). Voshell and Hilu (2014) proposed that *P. californica* is the result of hybridization between *P. arundinacea* and an unknown closely related grass species.

investigate these hypotheses. Specifically, we hypothesize that within the genus *Phalaris* (1) polyploids should inhabit more extreme environments than their diploid congenors; (2) polyploids should have wider environmental niche breadths; (3a) polyploids should occupy distinct environmental niches from their diploid congenors, and (3b) polyploids should have significantly less niche overlap with their diploid congenors than their diploid congenors have with one another. We selected environmental variables that are known to be important correlates of plant species distributions at a global scale and are changing due to anthropogenic activities (Franklin, 2009; Visser et al., 2014) and used these to determine differences in niche dimensions (means, extremes, breadth and overlap) between diploid and polyploid *Phalaris* species.

The notion that polyploids may be more tolerant of stressful conditions (including drought, cold, heat, and low-nutrient conditions) than their parent species may be a consequence of the genomic changes after polyploidization (Hagerup, 1932; Grant, 1981; te Beest et al., 2012). Drought tolerance, specifically, may arise from the immediate increase in cell size that accompanies WGD (Stebbins, 1971; Kondorosi et al., 2000), and the concomitant reduction in transpiration rates as a result of there being a lower density of guard cells (which however are larger; (Li et al., 1996; Maherali et al., 2009; Van Laere et al., 2011; te Beest et al., 2012). Polyploids have been found to be more tolerant of water stress (Li et al., 2009; Liu et al., 2011; Van Laere et al., 2011; Manzaneda et al., 2012) and have greater fitness in drier environments (Ramsey, 2011). Although polyploids have been shown to occur in drier environments (Levin, 2002; Martin and Husband, 2009; Treier et al., 2009; Ramsey, 2011; Manzaneda et al., 2012), there is also counter-evidence (Martin and Husband, 2009; Laport et al., 2012). In contrast to drought tolerance, tolerance of extremely high temperatures has been relatively little investigated from a biogeographical perspective (Martin and Husband, 2009), but there is some experimental evidence suggesting that polyploids have higher tolerance to extremely high temperatures (Waines, 1994; Soliman et al., 2012). At the other extreme, a greater tolerance to extreme cold has been suggested (Hagerup, 1932; te Beest et al., 2012), but with equivocal support for this hypothesis (Wit, 1958; Tyler et al., 1978; Lachmuth et al., 2010; Liu et al., 2011). Finally, polyploids might tolerate low nutrient soils better than diploids (Rohweder, 1937; Noguti et al., 1940; Schlaepfer et al., 2010; te Beest et al., 2012). However, this argument does not hold for P-poor soils because polyploids have a greater requirement for this element for nucleic material (Lewis, 1985, Šmarda et al., 2013).

Greater tolerance to extreme environmental conditions may also permit polyploids to occupy a wider range of environmental conditions than their parent species because greater tolerance allows a species to expand into these previously unoccupied environments (Stebbins, 1950; Soltis et al., 2010; te Beest et al., 2012). A wider niche breadth may also arise from the greater genetic diversity that polyploids may have as a result of hybridization and/or multiple origins of polyploidy, which may allow for greater phenotypic diversity within and between populations of polyploid species (Stebbins, 1950; Levin, 2002; Hahn et al., 2012; te Beest et al., 2012; Madlung, 2013). Genetic changes, gene expression changes, epigenetic silencing, subfunctionalization, and direct cellular effects may also allow for a wider genic response and, ultimately, phenotypic diversity and plasticity in polyploids (Stebbins, 1950; Levin, 2002; te Beest et al., 2012; Madlung and Wendel, 2013). In addition, the genetic, epigenetic, and physiological changes associated with WGD may also have the effect of producing a polyploid species with a distinctly different physiological tolerance than that of its parent species.

Because polyploid species arose from diploid progenitors, they are closely related and may therefore exhibit phylogenetic niche conservatism (PNC; Wiens et al., 2010). PNC has been

found to be widespread within the grasses (Edwards and Smith, 2010). However, PNC justifies the comparison of polyploids to congeneric diploids, even if the specific diploid progenitors of the polyploid species are not known. Moreover because of the difficulty involved in matching diploid progenitors with the relevant polyploids species, other studies have controlled for phylogenetic relatedness without explicitly identifying diploid progenitors (e.g., Martin and Husband, 2009). PNC also provides the rationale for hypothesis 3b, because we expect that closely related species should retain similar niches, but that polyploidization perhaps provides the opportunity for ecological differentiation of polyploid species. Therefore, we might expect closely related diploid species to have more similar niches to one another than they do relative to their closely related polyploid species.

MATERIALS AND METHODS

***Distribution data*—**The genus *Phalaris* is thought to have a Mediterranean origin, with a number of species having a mostly Mediterranean distribution, but the New World $x = 7$ is naturally restricted to the Americas (Voshell et al., 2011; Voshell and Hilu, 2014; Appendix S2, see online Supplemental Data). We searched the literature and a number of online herbaria for distribution data (online Appendix S3) for all 19 *Phalaris* species and found at least 10 records for 14 species. Using data from a number of different sources helps to reduce sampling biases that may exist in any one database (e.g., Newbold, 2010). Distribution records not occurring on land (i.e., misspecification of coordinates or lack of precision causing points to occur in large water bodies), fossil records and records with latitude or longitude assigned as zero were removed (Yesson, et al., 2007). Records for which coordinates were determined using place names on labels (GBIF georeferencing protocol "map estimate") were also removed. For each species, records were checked with respect to each of the environmental variables included in the analyses for outliers using box plots generated in the R statistical software (R Development Core Team, 2014). Using this approach, outliers were identified as records that were more than 1.5 times away from the first and third quartiles as these were from the median, i.e., outlier ≈ median ± [abs(quartile − median) × 1.5]. Outliers were then manually checked for possible georeferencing errors and corrected based on locality descriptions or deleted if this was not possible.

Two species, the diploid *P. rotgesii* and hexaploid *P. caesia*, are recognized by most grass taxonomists as subspecies of the tetraploid *P. arundinacea* (e.g., Clayton et al., 2002 onward) and are therefore usually recorded under this species' name. *Phalaris rotgesii* is known to be endemic to the Mediterranean islands of Corsica and Sardinia (Baldini, 1995), and we found eight records on Corsica. However, we made the assumption that *P. rotgesii* can occur anywhere on Corsica and Sardinia, except in environmental grid cells above 1700 m a.s.l. based on the distribution of this species described by Baldini (1995) (Appendix S2 H). We excluded *P. caesia* because Baldini (1995) provides a much less circumscribed distribution for this species (France, Spain, Portugal, Turkey, Lebanon, eastern and southern Africa), and we only found two records for it. However, the remaining records for *P. arundinacea* within the native range of *P. caesia* could actually be the latter species. We tested whether the inclusion of *P. rotgesii* and *P. arundinacea* affected results at the whole-genus level, detailed in the relevant sections.

Our focus was on the species' native ranges, which were determined using information from a number of sources (online Appendix S4). However, sources were sometimes in disagreement about the native/alien status of a species, and we therefore categorized each region as either "status 1" (no conflict among references), or "status 2" (native status given by any of the references, even if conflicted by another reference). We ran all the analyses, once with both status 1 and 2 records, and once with only status 1 records; however, we found there was little difference between these results (online Appendices S5 and S6) and report only the first set of analyses in the results.

***Environmental data*—**We used three temperature- and two precipitation-related variables with a spatial resolution of 2.5 min from WORLDCLIM (Hijmans et al., 2005): mean annual temperature (T mean), minimum temperature of the coldest month (T min), maximum temperature of the warmest month (T max), mean annual precipitation (Prec), and precipitation seasonality (Prec Seas). We also used three global soil data sets: a soil hydrological moisture index, with 0 representing extreme water stress and 1 representing no water stress (Soil Moist; 2.5 min. resolution; Trabucco and Zomer, 2010); one representing N content in the topsoil (Soil N; 5 min. spatial resolution; Batjes, 1997) and the other of potential soil P retention capacity (Phos; 5 min. spatial resolution; Batjes, 2011).

***Niche means, extremes, and breadths*—**For each of the 13 *Phalaris* species, we extracted data for the aforementioned environmental variables within the R environment (R Development Core Team, 2014) and removed all duplicate occurrences. To depict the frequency of each species' occurrences across the full gradient of each environmental variable, we used kernel density plots, which provide a standardized density (from 0 to 1) of species occurrences.

To test the hypothesis that polyploids occupy more extreme environments (hypothesis 1), we examined differences between species in their means and extremes (5th and 95th percentiles) of the sample of data corresponding to each species for each environmental variable. In the results, we focused only on either the lower or upper extreme, depending on the environmental variable in question. This is because, arguably, only particular extreme types (lower or upper) will be biologically meaningful. For example, the lower extreme of T min indicates a species' tolerance to extreme cold (Humphreys and Linder, 2013), but the upper extreme of this variable is probably not biologically meaningful. To test the hypothesis that polyploids have wider niche breadth (hypothesis 2), we used the geographic location of occurrences for each species to extract environmental variable values from the aforementioned spatial environmental layers and calculated the standard deviation of these data (for each environmental variable) as a measure of niche breadth.

We used two different approaches to statistically test the first two hypotheses. First, we used a bootstrapping approach, which helps to account for sampling biases in the distribution data (Ruxton and Neuhäuser, 2013). For this approach, we pooled data for two species and selected from this pooled data set two samples of equivalent size to the original sample sizes of each species and did this for each pairwise combination of all 13 species. (There are 78 pairwise combinations in total. However, in the results, we focus on the 16 pairwise combinations represented by species only within the four major lineages. We focused on these four lineages as a means of accounting for phylogenetic niche conservatism [Wiens et al., 2010], but results for all pairwise combinations are provided in online Appendices S7–S11.) From these samples, we calculated the measure of interest (i.e., the means, the 5th and 95th percentiles, or the standard deviations) for each species and calculated the difference, $\Delta_{\mathrm{sim},j}$, between the two species, repeating this process 9999 times. *P* values were calculated as:

$$P = \frac{1 + \sum_{j=1}^{B} I\left(\Delta_{\mathrm{sim},\,j} > \Delta_{\mathrm{obs}}\right)}{10000},$$

where Δ_{obs} is the actual difference between the two species, and I is an indicator function that is either one when the argument in parentheses is true or zero when false (Ruxton and Neuhäuser, 2013). The *P* value calculated as above will approach the "true" *P* value with increasing sample size, but will not be exactly repeatable because samples are selected at random (Ruxton and Neuhäuser, 2013). Confidence intervals around a particular significance level can be estimated (Gandy, 2009), which we did using a 5% significance level and using the R package "simctest" (Gandy, 2009). We do not report confidence intervals for significance, but take the conservative approach of only regarding results where the upper confidence interval ≤ 5%.

Our second approach to test for differences between polyploid and diploid species was to use linear models within the R environment (R Development Core Team, 2014). We used the measure of interest as the response variable and ploidy level of species as a predictor variable. Although this approach does not account for statistical nonindependence of species due to shared evolutionary history, alternatives such as phylogenetic generalized least squares regression assume species are statistically independent replicates (Stone et al., 2011). Allopolyploid species may have experienced relatively recent hybridization and thus are perhaps still experiencing gene flow between diploid and tetraploid individuals. Therefore, allopolyploid species are not always statistically independent of one another from a genetic perspective. Given this uncertainty, we have opted for linear models as the simplest approach for examining interspecific differences in niche measures. We also tested whether excluding *P. rotgesii* and *P. arundinacea* affected these results, because of problems ascribing precise species identities for geographic records.

***Niche overlap*—**We used Schoener's *D* (Schoener, 1970), a measure of niche similarity, to test the hypotheses about ploidy level and niche overlap (hypotheses 3a and 3b), using the methods of Broennimann et al. (2012). This required selecting a species' background area to characterize the environmental conditions available for species to occupy, as well as a "global background". We defined the boundaries of these two background areas on political boundaries, using the third level of the World Geographical Scheme for Recording Plant Distributions (Biodiversity Information Standards [TDWG], 2007), because most sampling of herbarium specimens is recorded at a national level and defining the background area in such a manner better reflects sampling bias at a global scale. However, results using the terrestrial realms defined by Olson et al. (2001; Appendix S2) to define the background extent were comparable (Appendix S12). As species' background areas, we used the native continent, being either Eurasia or the Americas, with all TDWG regions in Eurasia in which there was an occurrence of any *Phalaris* species being combined to form the Eurasian background, and the same for the Americas. The global background area was defined as either both continents combined, in cases where one species was from Eurasia and the other from the Americas, or as the continent from which both species came.

Values for each environmental variable described above (except aggregated to 0.5° resolution) representing the available climate space were then extracted from the global background area and binned into 100 equal-sized intervals across the full gradient of each environmental variable. The occurrence values were binned into the same intervals, and both the background and occurrence interval data were converted to densities. Both densities were then smoothed and the occurrence densities corrected for the availability of environmental variable intervals using the smoothed background densities. Niche overlap (*D*) was calculated by summing the differences between two species in their occupancies of each environmental variable interval (Broennimann et al., 2012).

Statistical significance of *D* values was determined using both niche equivalency and niche similarity tests (Warren et al., 2008; Broennimann et al., 2012). The niche equivalency test requires pooling all the data for both species, randomly splitting these data into two data sets of the same sizes as the original two data sets, and thereafter calculating *D*. This process was repeated 100 times, and the null hypothesis of the two species' niches being equivalent was only rejected if the *D* statistic calculated using the true occurrences for each species did not fall within the 95th percentile of the simulated *D* values (Broennimann et al., 2012). The niche equivalency test is relatively conservative because it is unlikely for different species to have statistically equivalent niches (Glennon et al., 2014). Therefore, we also used the niche similarity test, which tells us whether two species' niches are distinguishable from one another given the variability of the environment within which the species are found. This is actually two tests, with the first starting with a random selection of points from the species' background areas equivalent to the number of occurrences as the second species in the pairwise comparison, thereby generating a random niche, and then using the observed niche values for the first species to calculate *D*, repeating this 100 times. If the observed *D* value of the first species was less than 95% of the simulated *D* values, then there was evidence of niche differentiation between the two species. The second test is the same as above, but instead using the observed occurrences of the second species to calculate *D* (Broennimann et al., 2012). We focused on the 16 within-lineage pairwise species combinations in the results, but results for the entire genus are provided in Appendices S13 and S14 (see Supplemental Data).

To test whether *D* was significantly different for diploid–diploid species comparisons in relation to diploid–tetraploid species comparisons (hypothesis 3b), we used linear mixed-effect models (i.e., ploidy comparison was used as a fixed factor, and only within-lineage comparisons were included in the model), while controlling for shared evolutionary history by including the lineage comparison (e.g., $x = 6$ vs. Arundinacea $x = 7$) as a random factor in the models. As for the niche means, extremes and breadth linear models, we also tested whether excluding *P. rotgesii* and *P. arundinacea* affected these results. Models were implemented in the R environment (R Development Core Team, 2014) using the package "nlme" (Pinheiro et al., 2014).

RESULTS

***Do polyploids occupy more extreme environments than diploids?*—**There was no consistent pattern of polyploids occupying more extreme environments than their most closely related diploid species at the whole-genus level, as determined using a linear model, and even after excluding the two Arundinacea $x = 7$ species (Figs. 2, 3; online Appendix S15). For pairwise species differences, we did find significant differences between diploid and polyploid species (Appendices S7–S11), but the majority of differences between diploids and tetraploids in niche extremes were significant, and in a biologically meaningful direction only for the temperature mean (T mean) (negative differences for lower extremes for 6/8 within-lineage comparisons and 21/36 entire-genus comparisons; Appendix S11 B), temperature minimum (T min) (negative difference for lower extremes for 17/36 entire-genus comparisons; Appendix S11 B), precipitation mean (Prec) (positive differences for upper extremes for 5/8 within-lineage comparisons and 17/36 entire-genus comparisons; Appendix S11 C), and the soil moisture index (Soil Moist) (positive differences for upper extremes for 5/8 within-lineage pairwise species comparisons and 17/36 entire-genus comparisons; Appendix S11 B).

For the Old World $x = 7$ species, the two tetraploid species, *P. minor* and *P. aquatica*, were never found to occupy more extreme environments than either of their most closely related diploid species, *P. paradoxa* and *P. coerulescens* (Figs. 4–6; Appendices S7–S9). However, relative to *P. coerulescens*, *P. minor* occupies regions that are drier and have lower soil phosphorous (Phos) (negative differences for mean values and lower niche extremes for Prec, Soil Moist; Appendices S7, S8). Relative to the diploid *P. paradoxa*, *P. minor* occupies regions that are colder, drier, and have lower Phos (negative difference for mean values and lower niche extremes of T mean, T min, and Phos; Figs. 4, 5; Appendices S7, S8). *Phalaris aquatica* occurs in regions that are wetter than the diploid *P. coerulescens* (positive differences for means and upper extremes of Prec and Soil Moist; Figs. 4, 6; Appendices S7, S9). *Phalaris aquatica* occurs in regions that are colder and wetter than the diploid *P. paradoxa* (negative differences for means and lower extremes of T mean and T min, and positive differences for means and upper extremes of Prec and Soil Moist; Figs. 4, 5; Appendices S7, S9).

For the Arundinacea $x = 7$ lineage, the tetraploid *P. arundinacea* occupied wetter (higher means and upper extremes for Prec and Soil Moist; Figs. 4, 6; Appendices S7, S9) and colder environments (negative differences for means and lower extremes of T mean and T min; Figs. 4, 5; Appendices S7, S8) than the diploid *P. rotgesii*.

Within the New World $x = 7$, the tetraploid *P. californica* does not occupy more extreme environments than any of the three diploid species in this lineage for any of the environmental variables investigated, except perhaps for Phos (higher mean values for Phos; Figs. 4–6; Appendices S7–S9). There is some evidence to suggest that it occupies wetter environments than *P. lemmonii* (higher upper extremes and mean values for Prec and Soil Moist; Figs. 4, 6; Appendices S7, S9), and perhaps *P. caroliniana* (higher mean for Prec and Soil Moist; Fig. 4; Appendix S7).

***Do polyploids have wider niche breadths than diploids?*—**There was no consistent pattern of tetraploids occupying a wider range of environmental conditions than diploids, even after excluding the two Arundinacea $x = 7$ species (Fig. 3; Appendix S15), and there was little evidence to support this hypothesis based on the individual diploid–tetraploid species comparisons (Fig. 7; Appendix S10, S11 D, see Supplemental Data). For within-lineage comparisons, the majority of diploid–tetraploid comparisons were both significant and positive for Prec, Soil Moist and Phos, (Prec: 5/8, Soil Moist: 4/8; Phos: 5/8; Fig. 6). For comparisons across the entire genus the same was true for T mean, T min, the percentage N in the topsoil (Soil N), and Phos

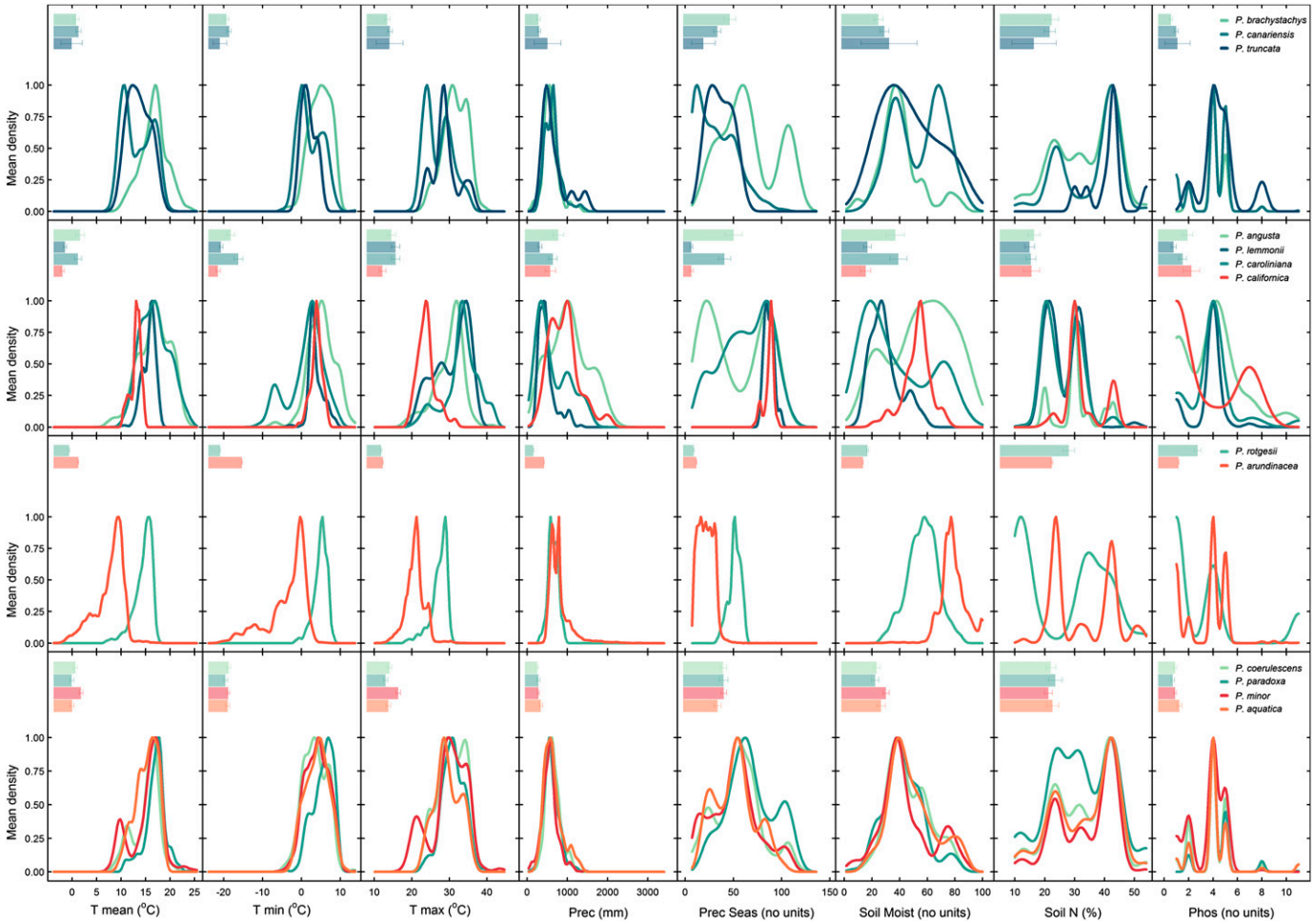

Fig. 2. Visual summary of environmental niches of all the *Phalaris* species analyzed, with species grouped into the four major lineages (x = 6, New World x = 7; Arundinacea x = 7 and Old World x = 7). For each environmental variable, we show the distribution of occurrences across the full environmental gradient (on the x-axis) as a kernel-density plot, represented by the colored lines (density of occurrences is scaled from zero to a maximum of one). In the top-left corner of each sub-image are bar plots representing niche breadths, which are the standard deviation of each environmental variable for each species. Error bars are 95% confidence intervals. Diploid species are shown in green to blue colors; polyploids are in yellow to red colors. Abbreviations for environmental variables are as follows: T mean = mean annual temperature, T min = minimum temperature of the coldest month, T max = maximum temperature of the warmest month, Prec = mean annual precipitation, Prec Seas = coefficient of variation of annual precipitation, Soil Moist = soil moisture index, Soil N = % N in the topsoil, Phos = potential soil P retention capacity).

(T mean: 14/36; T min: 17/36; Soil N: 14/36; Phos: 15/36; Appendix S11 D).

In the Old World x = 7, only for Soil Moist did both of the tetraploid species, *P. minor* and *P. aquatica* have significantly broader niches than their two most closely related diploid species, *P. coerulescens* and *P. paradoxa* (Figs. 2, 7; Appendix S10). *Phalaris minor* had a significantly broader niche for T mean, the temperature maximum (T max) and Soil Moist relative to both diploid species in this lineage, for Prec relative to *P. coerulescens*, and for T min and Phos relative to *P. paradoxa* (Fig. 7; Appendix S10). However, *P. minor* had a significantly narrower niche relative to the diploid *P. paradoxa* for Soil N (Fig. 7; Appendix S10). *Phalaris aquatica* had a broader niche relative to both diploid species for Prec, Soil Moist, and Phos, and for T min and T max relative to *P. paradoxa* (Fig. 7; Appendix S10). However, *P. aquatica* had a narrower niche relative to *P. coerulescens* for T mean, T max, and precipitation seasonality (Prec Seas) and relative to *P. paradoxa* for Prec Seas (Fig. 7; Appendix S10).

For the Arundinacea x = 7, the tetraploid *P. arundinacea* had a significantly broader niche than the diploid *P. rotgesii* for T mean, T min, T max, Prec, and Prec Seas, but a significantly narrower niche for Soil Moist, Soil N, and Phos (Fig. 7; Appendix S10). Interestingly, *P. arundinacea* did not have broader niches for four of the eight environmental variables than the two diploid Old World x = 7 species, *P. paradoxa* and *P. coerulescens* (Appendix S10), which are likely to be closely related to the one diploid progenitor of *P. arundinacea* (Fig. 1B).

For the New World x = 7, the tetraploid *P. californica* never had significantly broader niches than any of the three diploid species in this lineage (Fig. 7; Appendix S10). Relative to *P. lemmonii*, it had significantly broader niches for Prec and Phos and for Phos relative to *P. caroliniana*. However, it had a significantly narrower niche for six environmental variables in relation to *P. angusta*, five variables in relation to *P. caroliniana*, and three variables in relation to *P. lemmonii* (Fig. 7; Appendix S11 D).

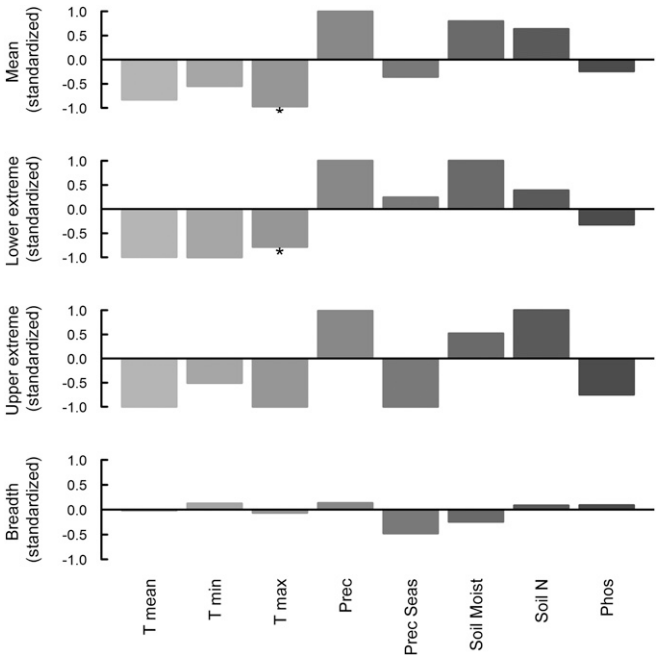

Fig. 3. Visual representation of linear model results with each niche measure (mean, lower extreme, upper extreme, and breadth) as response variable and ploidy level as a predictor (full results: Appendix S15). Bars represent slope coefficients from the models standardized by the maximum absolute value of each environmental variable to vary between −1 and 1. Significant results at the 5% level are indicated by an asterisk (*). See Fig. 2 for variable name abbreviations.

Do polyploids occupy different environmental niches than their diploid congenors?—We found that tetraploid species did not have equivalent niches to diploid species (significant differences in *D* values between species as measured using the niche equivalency test) for most pairwise comparisons for all environmental variables, but this was similar for diploid–diploid species comparisons (Fig. 8; Appendix S14). Similarly, for the niche similarity test, the majority of diploid–tetraploid comparisons were not significantly similar, except for Phos, and to a lesser degree, Soil N, but the pattern was the same for diploid-diploid comparisons (Fig. 8; Appendices S13, S14).

Do polyploids have significantly less niche overlap with their diploid congenors than their diploid congenors have with one another?—We found that diploid–diploid comparisons did not have lower niche overlap than diploid–tetraploid comparisons, except for T mean (Table 1), and even after excluding the two Arundinacea $x = 7$ species (Table 1), providing little support for this hypothesis.

DISCUSSION

Overall, we found little evidence to support the hypothesis that polyploidization creates species that have greater niche breadth, and/or different niches or can occupy more extreme environments than the diploid speicies. Given the well-documented genomic and physiological changes that take place during polyploidization, it is surprising that these known differences do not result in clear ecological differentiation between diploid and polyploid species. Rather, we found a much more nuanced pattern, with these hypotheses only having support for some pairwise species comparisons and only for a few environmental variables. Other cytobiogeographical studies have also failed to find evidence that polyploid species occupy more extreme environments (Tyler et al., 1978; Stutz and Sanderson, 1983; Gauthier et al., 1998; Hardy et al., 2000; Schönswetter et al., 2007; Martin and Husband, 2009; Laport et al., 2012; Glennon et al., 2014). Moreover, experimental approaches have failed to reveal polyploid species occupying wider niches (Stebbins and Dawe, 1987; Petit and Thompson, 1999; Martin and Husband, 2009; Glennon et al., 2012; Theodoridis et al., 2013; Harbert et al., 2014) or distinct niches from their lower-ploidy progenitors (Štěpánková, 2001; Baack and Stanton, 2005; Mandáková and Münzbergová, 2006; Glennon et al., 2012, 2014; Godsoe et al., 2013). These studies, in addition to our findings, suggest that these hypotheses are not universally upheld, therefore suggesting that more mechanistic studies will be required to understand how WGD influences ecological niches. For *Phalaris*, effects of polyploidization at a molecular and nuclear level have not been investigated and would be a first step in determining how genetic changes scale up to physiological processes and ultimately biogeographic distribution patterns.

Some cellular changes, such as an increase in overall cell size, may be an inevitable consequence of polyploidization (Speckmann et al., 1965; Stebbins, 1971; Masterson, 1994; Hodgson et al., 2010). Increased cell size results in direct physiological changes, such as a decrease in stomatal density (Li et al., 1996; Maherali et al., 2009), which can affect the water budget of plants and underpins the hypothesis that polyploids occupy drier environments than their diploid progenitors (te Beest et al., 2012). Currently, we do not know the relationship between cell size and ploidy level in *Phalaris*. However, if *Phalaris* follows the predicted trend of larger cell size for larger ploidy levels, the results provided here do not indicate that polyploids occupy drier environments than diploids. Rather, it appears that for *Phalaris*, the Mediterranean origin of the species suggests that the diploid species occupy drier conditions than the polyploid species. Thus, the evolutionary origin of the species may be more significant than the general patterns related to cell size. In the case of *Phalaris*, it appears that polyploidization has allowed species to become more competitive in moister habitats (e.g., *P. arundinacea*, *P. aquatica*, and *P. californica*; Baldini, 1995; Barkworth et al., 2007) and that *Phalaris* tetraploids occupy wetter environments than diploids (Figs. 3 and 5; Appendix S11A and C). However, we did not find general support for this hypothesis across all species (Fig. 3), and in fact, the pattern may be an artifact of *P. arundinacea* occupying much wetter environments than *P. rotgesii*, than any tetraploids vs. diploids in any of the other lineages (Appendices S9 and S15).

The origin of the parental diploids may influence the relationship between polyploidization and the hypothesized ecological responses. Autopolyploids are expected to have fewer genetic novelties than allopolyploids and, therefore, possibly lower heterozygosity, phenotypic plasticity, and niche flexibility (Parisod et al., 2010). In our study, all polyploid species are thought to be allopolyploids (Voshell et al., 2011; Jakubowski et al., 2013; Voshell and Hilu, 2014; Fig. 1B), although, all progenitors of *Phalaris* polyploids have not been identified (Fig. 1B). Allopolyploids may exhibit expression level dominance (one of either the paternal or maternal genes can determine overall gene expression), or homeolog expression

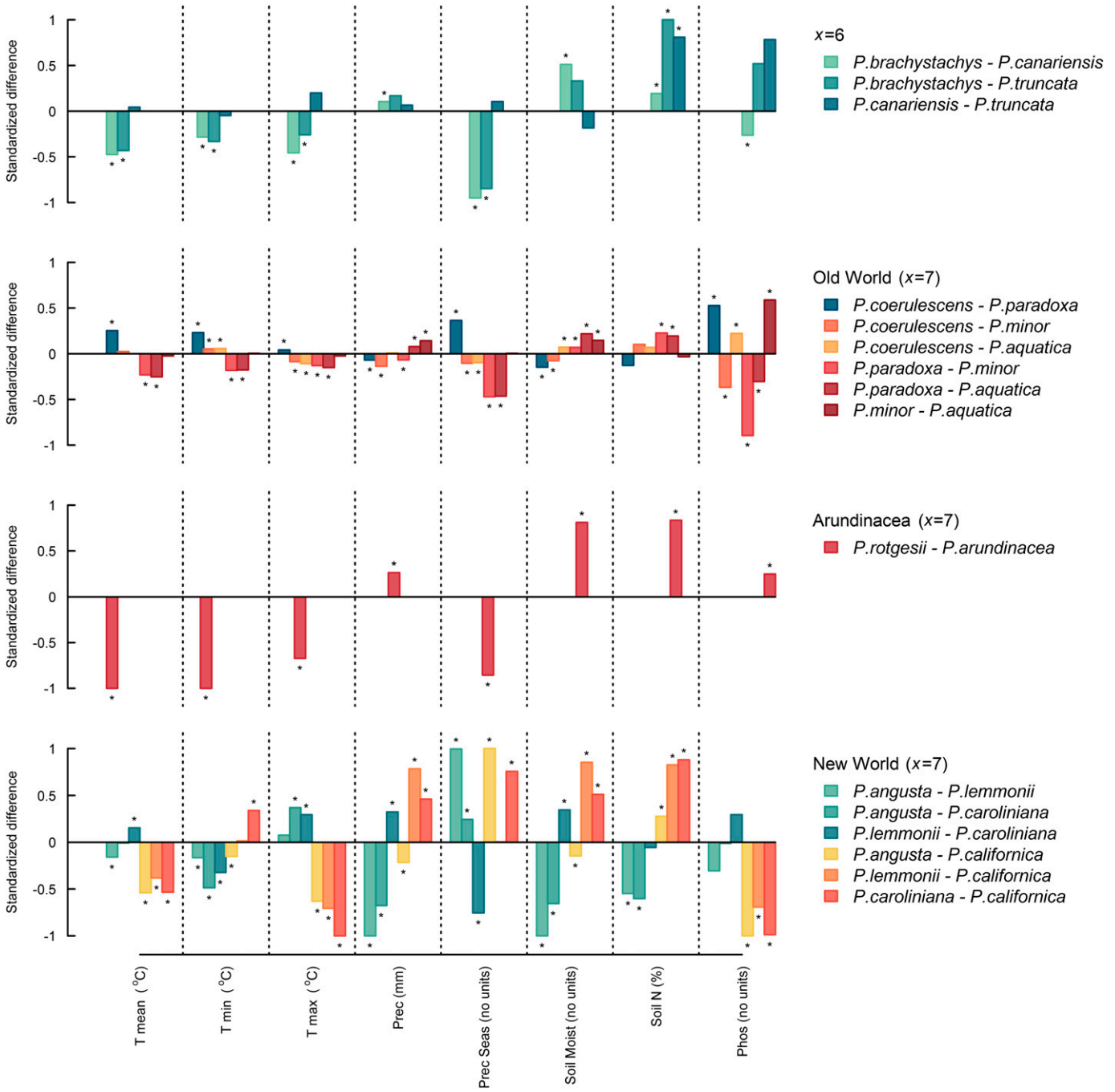

Fig. 4. Pairwise species differences in their means of all the environmental variables analyzed. Only pairwise comparisons for species within each of the four major lineages are shown, but the results for all species are provided as supplementary material (Appendix S7). Bars represent standardized (−1 to 1; values divided by the maximum absolute value of each variable) differences between species in their means. Green to blue represent pairwise comparisons between two diploid species. Yellow to red bars represent pairwise comparisons between tetraploid and diploid species. There was only one tetraploid–tetraploid comparison (*P. minor* and *P. aquatica*), which is represented by a dark red bar. Bootstrapped significance at a 5% level is indicated by an asterisk (*). See Fig. 2 for variable name abbreviations.

bias (preferential expression of one of either the paternal or maternal genes) (Madlung and Wendel, 2013), which can result in genes from one of the parental species being preferentially expressed. This can allow them to live in habitats that are unsuitable for their parental progenitors. In our study genus, *P. minor* is hypothesized to originate from *P. paradoxa* (Fig. 1B). *Phalaris minor* exhibited significant change in niche means and extremes and overlapped less in environmental niche space with *P. paradoxa*, than with its other diploid congenor, *P. coerulescens* (Figs. 2–5; Appendices S7–S9 and S13). These significant differences in niches between *P. minor* and *P. paradoxa*, but not between *P. minor* and

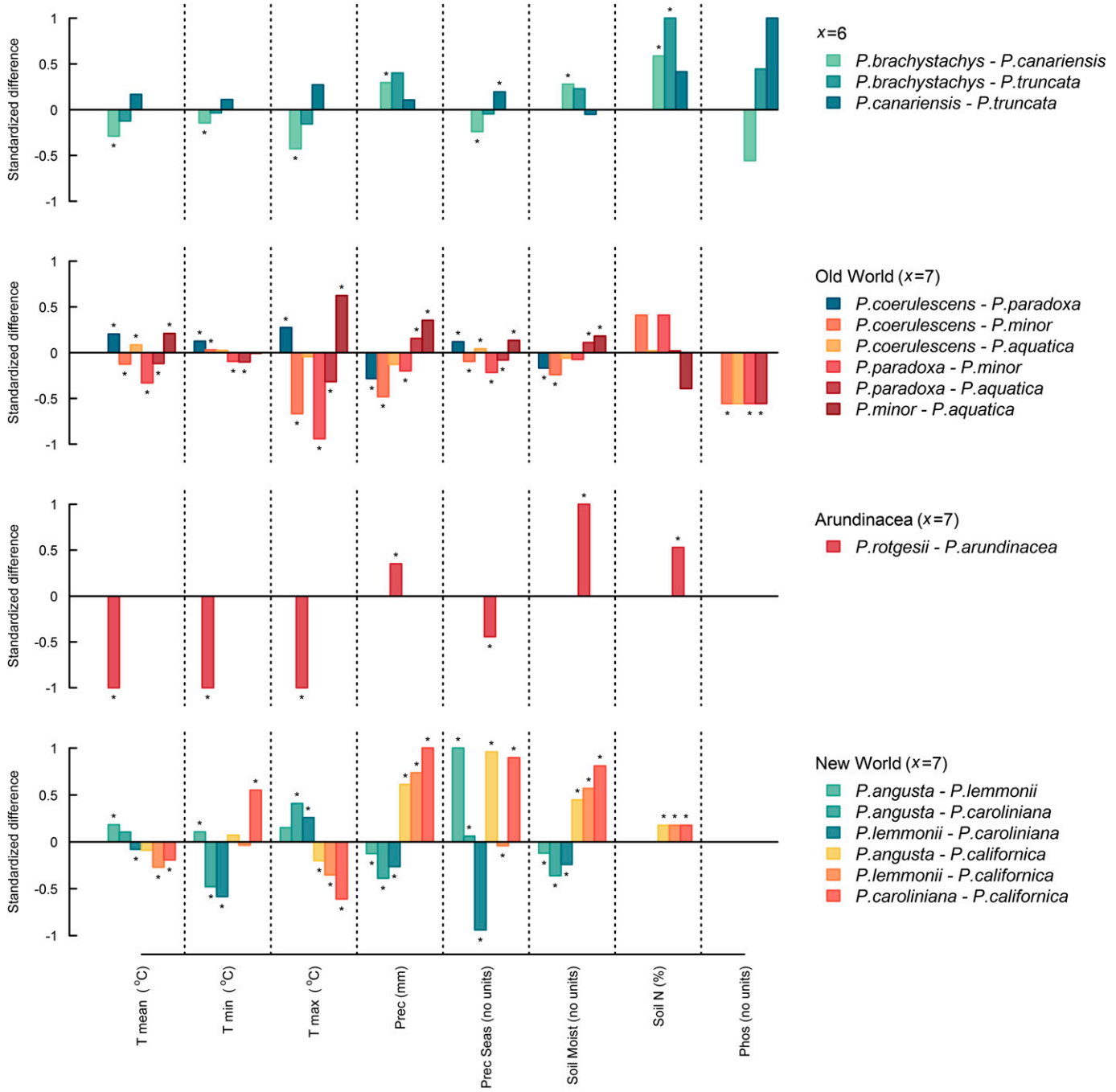

Fig. 5. Pairwise species differences in their lower extremes (5th percentile) of all the environmental variables analyzed. See Fig. 2 for variable names, Fig. 4 for figure interpretation. Results for all species are provided as supplemental data (Appendix S8).

P. coerulescens do not support preferential expression of *P. paradoxa* genes in *P. minor*, but suggest that *P. minor* occurs in different habitats to *P. paradoxa*.

One assumption of our hypotheses is that polyploidization will have a greater influence on ecological factors than phylogenetic niche conservatism (Martin and Husband, 2009). The grass family as a whole exhibits significant phylogenetic conservatism with respect to both temperature and precipitation niches (Edwards and Smith, 2010). Moreover, if phylogenetic niche conservatism is important, then novel polyploids and diploids will respond similarly to novel environments. Yet, there is little experimental evidence to suggest that polyploids enjoy a fitness advantage in novel environments relative to diploids (Madlung, 2013), although the strong association between polyploidy and invasiveness, and diploidy and rarity, for example, suggest there is a real fitness advantage to polyploidy (Pandit et al., 2011).

Studies such as ours that compare diploid and polyploid species that diverged a long time ago (in evolutionary terms) have been criticized because one cannot disentangle the effects of

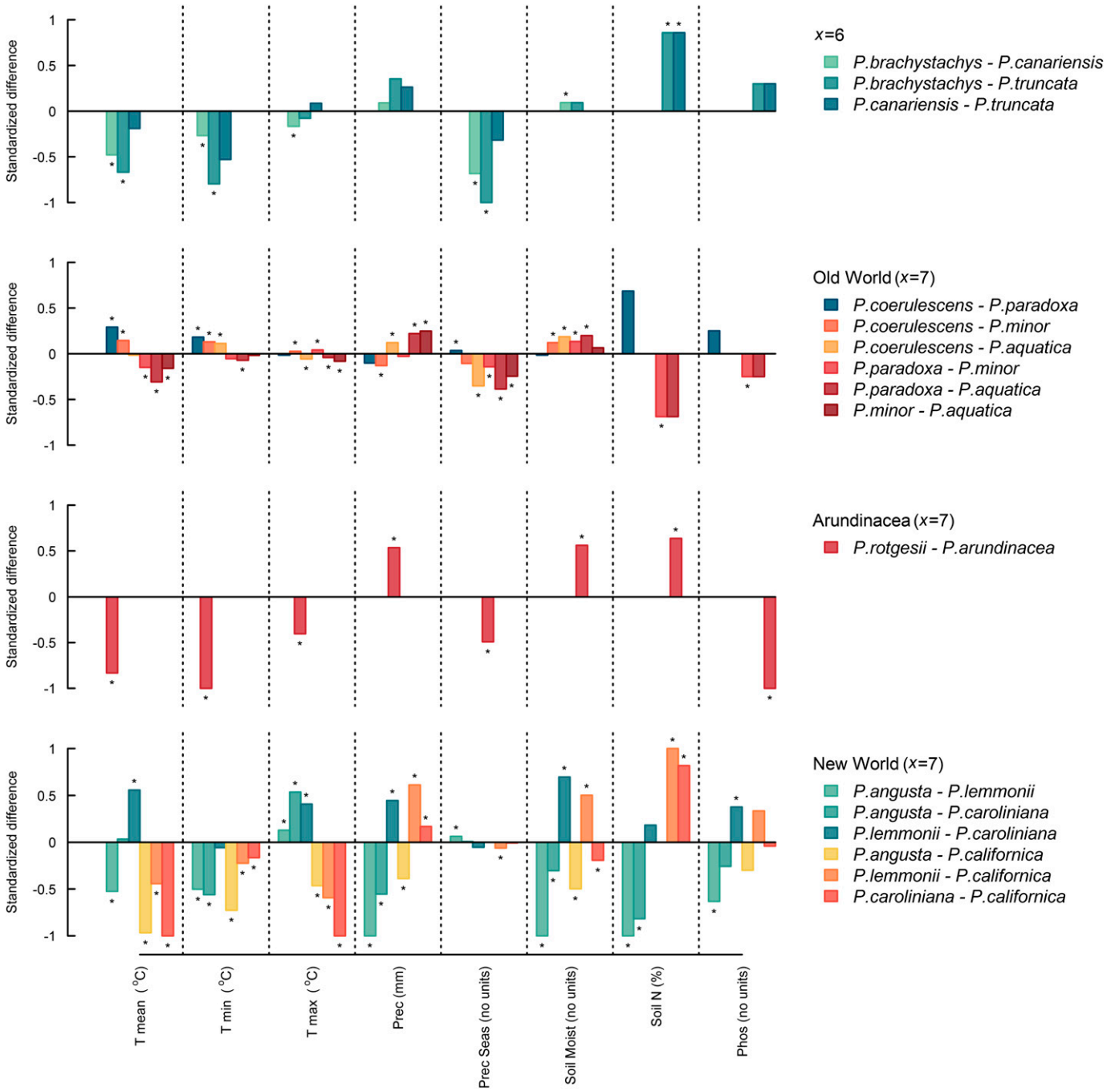

Fig. 6. Pairwise species differences in their upper extremes (95th percentile) for all the environmental variables analyzed. See Fig. 2 for variable names, Fig. 4 for figure interpretation. Results for all species are provided as supplemental data (Appendix S9).

polyploidization from "normal" genetic changes that might have occurred subsequent to polyploidization (Ramsey, 2011). However, these types of studies will generally "overestimate the phenotypic and ecological consequences of genome duplication" (Ramsey, 2011); yet our study found few differences between diploids and polyploids and thus further genetic differentiation postpolyploidization cannot explain our results. Ideally, correlative studies such as ours should be confirmed with experiments in which synthetic polyploids are generated (at least for the species whose ancestry is well known) and compared to the natural polyploids.

In addition, cytobiogeographical studies may be unable to identify the complete fundamental niche of species (Pearson and Dawson, 2003) because species may be physically prevented from access to regions that are climatically suitable for them by natural barriers (such as by intercontinental oceans as for our study; Pearson and Dawson, 2003). Sampling issues such as poor spatial resolution for important environmental

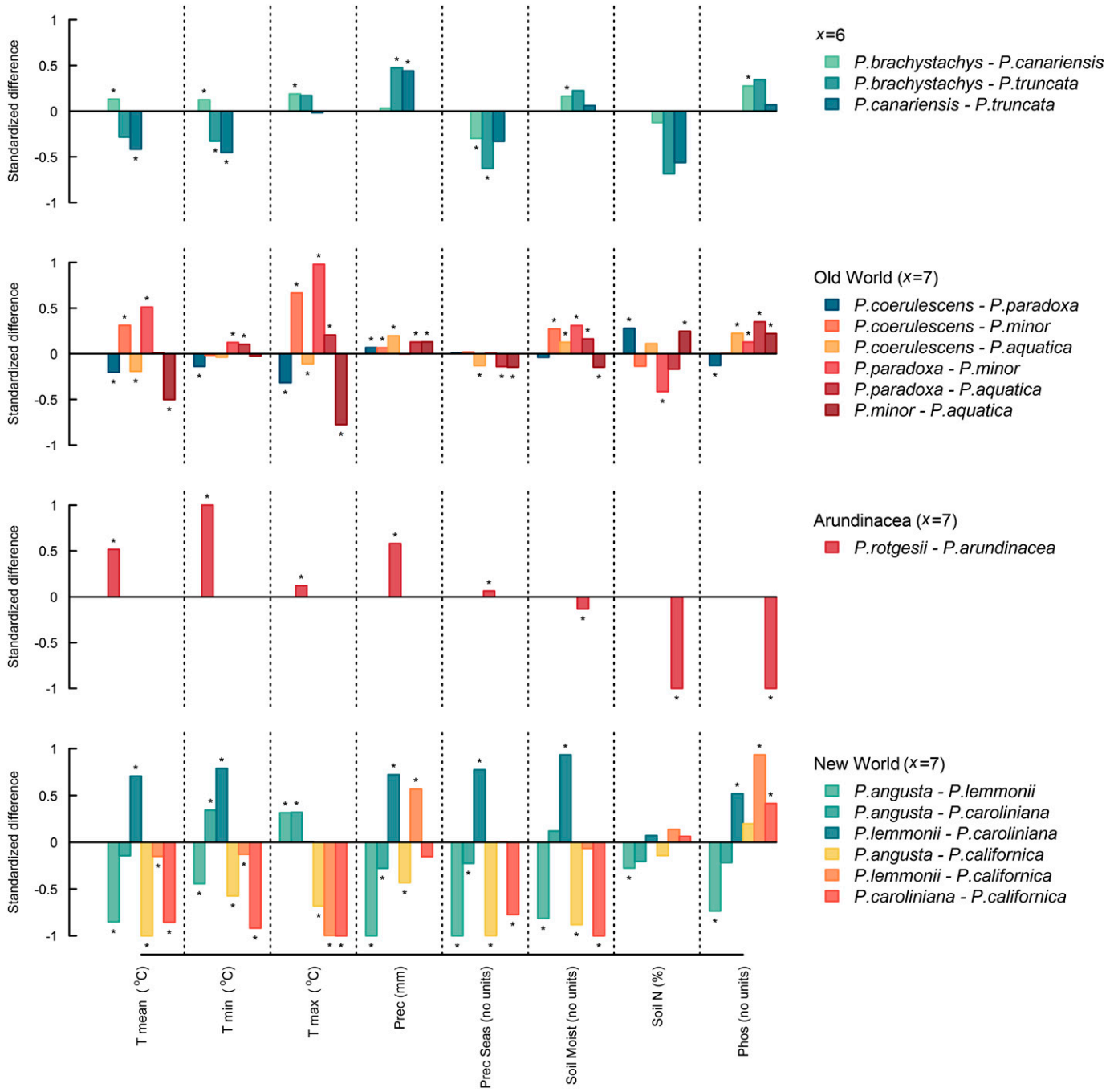

Fig. 7. Pairwise species differences in their breadths (standard deviations) for all the environmental variables analyzed. See Fig. 2 for variable names, Fig. 4 for figure interpretation. Results for all species are provided as supplemental data (Appendix S10).

data (Austin and Van Niel, 2011) and/or sampling biases may led to erroneous inferences about species' environmental niches (Newbold, 2010). In our study, we controlled for sampling bias by bootstrap sampling of species' occurrences (Ruxton and Neuhäuser, 2013). Notwithstanding these issues, cytobiogeographical studies provide a useful first approach to investigating climatic factors that might then be tested experimentally through common gardens and reciprocal transplant studies (Manzaneda et al., 2012). For *Phalaris*, we found that differences in niche overlap between diploid–diploid vs. diploid–tetraploid comparisons were only significant for mean temperature (Table 1). Thus, experimental manipulations of temperature in a greenhouse study may allow us to understand how genome duplication affects species characteristics affecting niche width.

Our results indicate overwhelmingly that our original predictions about the role of polyploidization and ecological niches were naïve. Rather, our results point out that the specifics of the polyploidization event, such as origin of the genera, parental origin, as well as unknown genetic factors and environmental

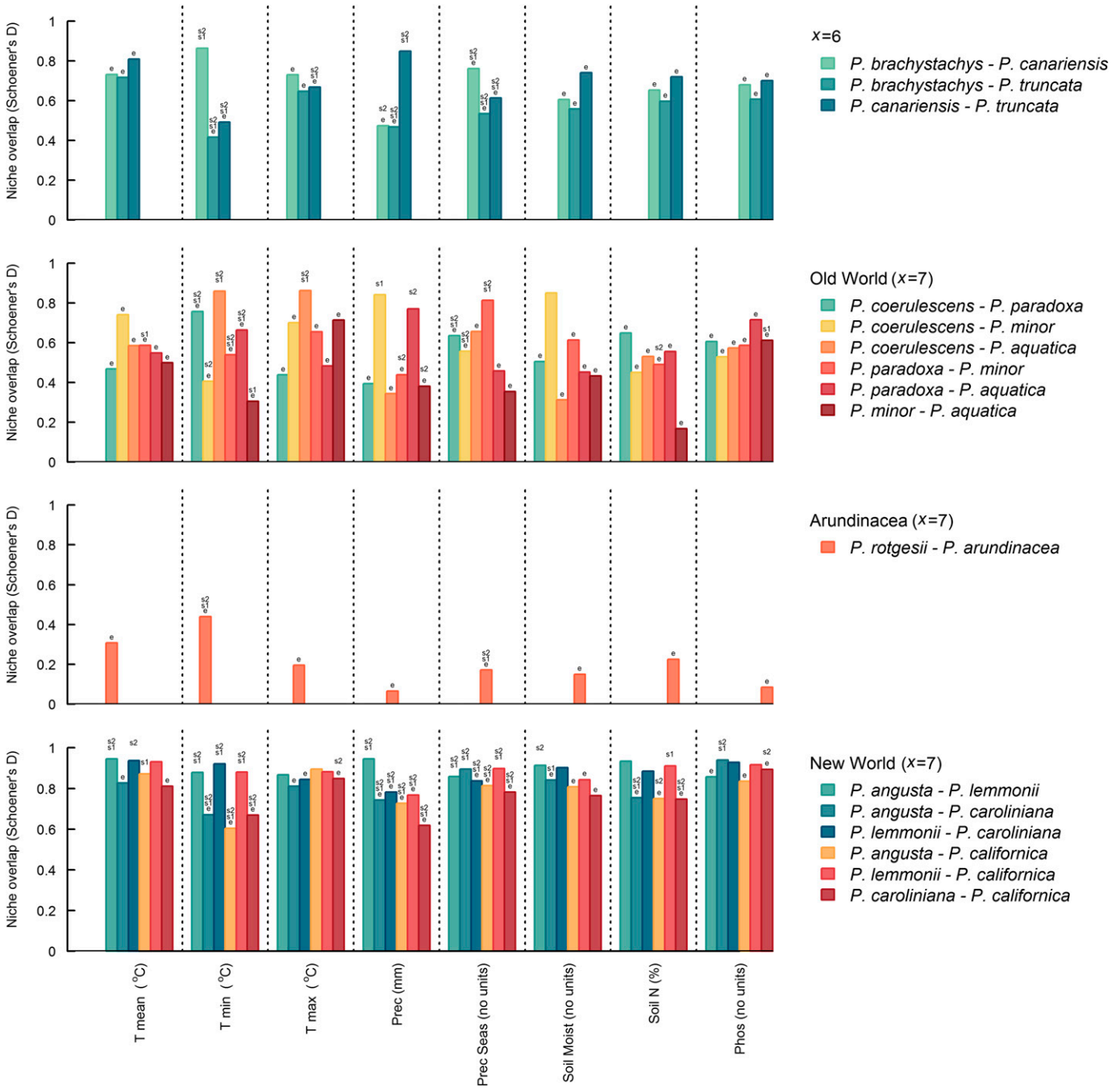

Fig. 8. Pairwise species niche overlap values (*D*) for a number of different environmental variables. Only pairwise comparisons for species within each of the four major lineages are shown, but the remaining results and counts of significant results are provided as supplemental data (Appendices S13 and S14). Bars represent *D* values with 0 representing no overlap between species and 1 representing complete overlap. Species pairs that had equivalent niches (significant niche equivalency tests) are indicated with an "e" above the relevant bar. Species pairs in which the first species had a significantly similar niche to the second species (as measured using the niche similarity test) are indicated with an "s1" above the relevant bar, and those where the second species had a significantly similar niche to the first species are indicated with an "s2". Variable names as for Fig. 2, colors as for Fig. 4.

factors influence individual species responses and niche widths. If we are to understand the role that WGD plays in the evolution and distribution of plant species, the way forward will require marrying the cytobiogeographical approach, with functional genomic approaches that document gene expression patterns under different controlled ecological conditions along with experimental approaches such as common gardens and reciprocal transplant studies for a well-studied phylogenetically controlled group of species in which there are multiple pairs of diploid–polyploid pairs. While the above program would be ambitious, the lack of generality found through cytobiogeographical approaches begs for more challenging experimental approaches to be applied (Madlung, 2013). Alternatively, neutral processes that can account for the observed lack of generality in the effects

TABLE 1. Linear mixed-effects model results in which niche overlap values for a number of different environmental variables (response variable) were predicted by ploidy comparison (i.e., diploid–diploid or diploid–tetraploid), while controlling for evolutionary history to a degree by including lineage comparison (e.g., $x = 6$ vs. $x = 6$) as a random factor. Results are shown for models with all species and for models excluding the Arundinacea $x = 7$ (*Phalaris rotgesii* and *P. arundinacea*). See Fig. 2 for variable names.

	All species			No Arundinacea $x = 7$		
Variable	Slope	t	P	Slope	t	P
T mean	**–0.16**	**–4.51**	**0.00**	**–0.10**	**–2.68**	**0.01**
T min	–0.04	–1.34	0.19	0.01	0.24	0.81
T max	–0.08	–2.52	0.01	–0.06	–1.24	0.22
Prec	0.01	0.19	0.85	0.02	0.43	0.67
Prec Seas	–0.08	–1.64	0.11	–0.10	–1.75	0.09
Soil Moist	–0.02	–0.51	0.61	0.01	0.49	0.62
Soil N	0.01	0.27	0.79	0.07	1.46	0.15
Phos	0.04	1.27	0.21	–0.05	–0.81	0.42

Notes: The slope represents the differences between diploid–diploid and diploid–tetraploid pairs (i.e., a lower value indicates less niche overlap between diploid–tetraploid pairs than diploid–diploid pairs). Also shown are associated P values ($P \leq 0.05$ in bold).

of polyploidization on species' niches need to be further investigated (Meyers and Levin, 2006).

LITERATURE CITED

AUSTIN, M. P., AND K. P. VAN NIEL. 2011. Improving species distribution models for climate change studies: Variable selection and scale. *Journal of Biogeography* 38: 1–8.

BAACK, E. J., AND M. L. STANTON. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): Niche differentiation and tetraploid establishment. *Evolution* 59: 1936–1944.

BALDINI, R. M. 1995. Revision of the genus *Phalaris* L. (Gramineae). *Webbia* 49: 265–329.

BARKWORTH, M. E., K. M. CAPELS, S. LONG, L. K. ANDERTON, AND M. B. PIEP. 2007. Flora of North America, vol. 24: Bambusoideae, Ehrhartoideae, and Pooideae [online]. Website http://herbarium.usu.edu/webmanual [accessed 30 January 2014].

BATJES, N. H. 1997. A world dataset of derived soil properties by FAO–UNESCO soil unit for global modelling. *Soil Use and Management* 13: 9–16.

BATJES, N. H. 2011. Global distribution of soil phosphorus retention potential [online]. Website: http://www.isric.org/data/global-assessment-soil-phosphorus-retention-potential [accessed 17 March 2014].

BIODIVERSITY INFORMATION STANDARDS (TDWG). 2007. World geographical scheme for recording plant distributions [online]. Website http://www.tdwg.org/standards/109/ [accessed 30 January 2014].

BROENNIMANN, O., M. C. FITZPATRICK, P. B. PEARMAN, B. PETITPIERRE, L. PELLISSIER, N. G. YOCCOZ, W. THUILLER, ET AL. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography* 21: 481–497.

CLAYTON, W. D., M. S. VORONTSOVA, K. T. HARMAN, AND H. WILLIAMSON. 2002 onward [continuously updated]. World grass species: Synonymy. Website http://www.kew.org/data/grasses-syn.html [accessed 30 January 2014].

EDWARDS, E. J., AND S. A. SMITH. 2010. Phylogenetic analyses reveal the shady history of C_4 grasses. *Proceedings of the National Academy of Sciences, USA* 107: 2532–2537.

FOWLER, N. L., AND D. A. LEVIN. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *American Naturalist* 124: 703–711.

FRANKLIN, J. 2009. Mapping species distributions: Spatial inference and prediction. Cambridge University Press, Cambridge, UK.

GANDY, A. 2009. Sequential implementation of Monte Carlo tests with uniformly bounded resampling risk. *Journal of the American Statistical Association* 104: 1504–1511.

GAUTHIER, P., R. LUMARET, AND A. BÉDÉCARRATS. 1998. Genetic variation and gene flow in alpine diploid and tetraploid populations of Lotus (*L. alpinus* (D.C.) Schleicher/*L. corniculatus* L.). I. Insights from morphological and allozyme markers. *Heredity* 80: 683–693.

GLENNON, K. L., L. J. RISSLER, AND S. A. CHURCH. 2012. Ecogeographic isolation: A reproductive barrier between species and between cytotypes in *Houstonia* (Rubiaceae). *Evolutionary Ecology* 26: 909–926.

GLENNON, K. L., M. E. RITCHIE, AND K. A. SEGRAVES. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17: 574–582.

GODSOE, W., M. A. LARSON, K. L. GLENNON, AND K. A. SEGRAVES. 2013. Polyploidization in *Heuchera cylindrica* (Saxifragaceae) did not result in a shift in climatic requirements. *American Journal of Botany* 100: 496–508.

GRANT, V. 1975. Genetics of flowering plants. Columbia University Press, New York, New York, USA.

GRANT, V. 1981. Plant speciation, 2nd ed. Columbia University Press, New York, New York, USA.

HAGERUP, O. 1932. Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. *Hereditas* 16: 19–40.

HAHN, M. A., Y. M. BUCKLEY, AND H. MÜLLER-SCHÄRER. 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. *Ecology Letters* 15: 947–954.

HARBERT, R. S., A. H. D. BROWN, AND J. F. DOYLE. 2014. Climate niche modeling in the perennial *Glycine* (Leguminosae) allopolyploid complex. *American Journal of Botany* 101: 710–721.

HARDY, O. J., S. VANDERHOEVEN, M. DE LOOSE, AND P. MEERTS. 2000. Ecological, morphological and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist* 146: 281–290.

HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

HODGSON, J. G., M. SHARAFI, A. JALILI, S. DÍAZ, G. MONTSERRAT-MARTÍ, C. PALMER, B. CERABOLINI, ET AL. 2010. Stomatal vs. genome size in angiosperms: The somatic tail wagging the genomic dog? *Annals of Botany* 105: 573–584.

HUMPHREYS, A. M., AND H. P. LINDER. 2013. Evidence for recent evolution of cold tolerance in grasses suggests current distribution is not limited by (low) temperature. *New Phytologist* 198: 1261–1273.

JACKSON, S., AND Z. J. CHEN. 2010. Genomic and expression plasticity of polyploidy. *Current Opinion in Plant Biology* 13: 153–159.

JAKUBOWSKI, A. R., M. D. CASLER, AND R. D. JACKSON. 2013. Genetic evidence suggests a widespread distribution of native North American populations of reed canarygrass. *Biological Invasions* 15: 261–268.

JIAO, Y., N. J. WICKETT, S. AYYAMPALAYAM, A. S. CHANDERBALI, L. LANDHERR, P. E. RALPH, L. P. TOMSHO, ET AL. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* 473: 97–100.

KONDOROSI, E., F. ROUDIER, AND E. GENDREAU. 2000. Plant cell-size control: Growing by ploidy? *Current Opinion in Plant Biology* 3: 488–492.

LACHMUTH, S., W. DURKA, AND F. M. SCHURR. 2010. The making of a rapid plant invader: Genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*. *Molecular Ecology* 19: 3952–3967.

LAPORT, R. G., R. L. MINCKLEY, AND J. RAMSEY. 2012. Phylogeny and cytogeography of the North American creosote bush (*Larrea tridentata*, Zygophyllaceae). *Systematic Botany* 37: 153–164.

LEVIN, D. A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.

LEVIN, D. A. 2000. The origin, expansion, and demise of plant species. Oxford University Press, New York, New York, USA.

LEVIN, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York, New York, USA.

LEWIS, W. H. 1980. Polyploidy in species populations. *In* W. H. Lewis [ed.], Polyploidy, basic life sciences, 103–144. Plenum Press, New York, New York, USA.

LEWIS, W. M. JR. 1985. Nutrient scarcity as an evolutionary cause of haploidy. *American Naturalist* 125: 692–701.

LI, W., D. K. BISWAS, H. XU, C. XU, X. WANG, J. LIU, AND G. JIANG. 2009. Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Functional Plant Biology* 36: 783–792.

LI, W.-L., G. P. BERLYN, AND P. M. S. ASHTON. 1996. Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). *American Journal of Botany* 83: 15.

LIND-HALLDÉN, C., C. HALLDÉN, AND T. SÄLL. 2002. Genetic variation in *Arabidopsis suecica* and its parental species *A. arenosa* and *A. thaliana. Hereditas* 136: 45–50.

LIU, S., S. CHEN, Y. CHEN, Z. GUAN, D. YIN, AND F. CHEN. 2011. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulturae* 127: 411–419.

LIU, Z., AND K. L. ADAMS. 2007. Expression partitioning between genes duplicated by polyploidy under abiotic stress and during organ development. *Current Biology* 17: 1669–1674.

LYNCH, M., AND A. FORCE. 2000. The probability of duplicate gene preservation by subfunctionalization. *Genetics* 154: 459–473.

MADLUNG, A. 2013. Polyploidy and its effect on evolutionary success: Old questions revisited with new tools. *Heredity* 110: 99–104.

MADLUNG, A., AND J. F. WENDEL. 2013. Genetic and epigenetic aspects of polyploid evolution in plants. *Cytogenetic and Genome Research* 140: 270–285.

MAHERALI, H., A. E. WALDEN, AND B. C. HUSBAND. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184: 721–731.

MANDÁKOVÁ, T., AND Z. MÜNZBERGOVÁ. 2006. Distribution and ecology of cytotypes of the *Aster amellus* aggregates in the Czech Republic. *Annals of Botany* 98: 845–856.

MANZANEDA, A. J., P. J. REY, J. M. BASTIDA, C. WEISS-LEHMAN, E. RASKIN, AND T. MITCHELL-OLDS. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* 193: 797–805.

MARTIN, S. L., AND B. C. HUSBAND. 2009. Influence of phylogeny and ploidy on species ranges of North American angiosperms. *Journal of Ecology* 97: 913–922.

MASTERSON, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. *Science* 264: 421–424.

MCINTYRE, P. J. 2012. Polyploidy associated with altered and broader ecological niches in the *Claytonia perfoliata* (Portulacaceae) species complex. *American Journal of Botany* 99: 655–662.

MEYERS, L. A., AND D. A. LEVIN. 2006. On the abundance of polyploids in flowering plants. *Evolution* 60: 1198–1206.

NEWBOLD, T. 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. *Progress in Physical Geography* 34: 3–22.

NOGUTI, Y., H. OKA, AND T. ÔTUKA. 1940. Studies on the polyploidy in *Nicotiana* induced by the treatment with colchicine. II. Growth rate and chemical analysis of diploid and its autotetraploid in *Nicotiana rustica* and *N. tabacum. Japanese Journal of Botany* 10: 343–364.

OLSON, D. M., E. DINERSTEIN, E. D. WIKRAMANAYAKE, N. D. BURGESS, G. V. N. POWELL, E. C. UNDERWOOD, J. A. D'AMICO, ET AL. 2001. Terrestrial ecoregions of the world: A new map of life on Earth. A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* 51: 933–938.

OSBORN, T. C., J. C. PIRES, J. A. BIRCHLER, D. L. AUGER, Z. JEFFERY CHEN, H.-S. LEE, L. COMAI, ET AL. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* 19: 141–147.

PANDIT, M. K., M. J. O. POCOCK, AND W. E. KUNIN. 2011. Ploidy influences rarity and invasiveness in plants. *Journal of Ecology* 99: 1108–1115.

PARISOD, C., R. HOLDEREGGER, AND C. BROCHMANN. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist* 186: 5–17.

PEARSON, R. G., AND T. P. DAWSON. 2003. Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography* 12: 361–371.

PETIT, C., AND J. D. THOMPSON. 1999. Species diversity and ecological range in relation to ploidy level in the flora of the Pyrenees. *Evolutionary Ecology* 13: 45–65.

PINHEIRO, J., AND D. BATES, S. DEBROY, D. SARKAR, AND R DEVELOPMENT CORE TEAM. 2014. nlme: Linear and nonlinear mixed effects models. R package version 3.1-117. Website http://cran.r-project.org/web/packages/nlme/ [accessed 30 January 2014].

R DEVELOPMENT CORE TEAM. 2014. R: A language and environment for statistical computing. Version R 3.1.0. Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/.

RAMSEY, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences, USA* 108: 7096–7101.

ROHWEDER, H. 1937. Versuch zur Erfassung der mengenmässigen Bedeckung des Darss und Zingst mit polyploiden Pflanzen. *Planta* 27: 500–549.

RUXTON, G. D., AND M. NEUHÄUSER. 2013. Improving the reporting of *P*-values generated by randomization methods. *Methods in Ecology and Evolution* 4: 1033–1036.

SAMPOUX, J.-P., AND C. HUYGHE. 2009. Contribution of ploidy-level variation and adaptive trait diversity to the environmental distribution of taxa in the "fine-leaved fescue" lineage (genus *Festuca* subg. *Festuca*). *Journal of Biogeography* 36: 1978–1993.

SCHLAEPFER, D. R., P. J. EDWARDS, AND R. BILLETER. 2010. Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: A common garden comparison of ploidy levels. *Oecologia* 163: 661–673.

SCHOENER, T. W. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* 51: 408–418.

SCHÖNSWETTER, P., M. LACHMAYER, C. LETTNER, D. PREHSLER, S. RECHNITZER, D. S. REICH, M. SONNLEITNER, ET AL. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* 120: 721–725.

ŠMARDA, P., M. HEJCMAN, A. BŘEZINOVÁ, L. HOROVÁ, H. STEIGEROVÁ, F. ZEDEK, P. BUREŠ, ET AL. 2013. Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytologist* 200: 911–921.

SOLIMAN, W. S., M. FUJIMORI, K. TASE, AND S. SUGIYAMA. 2012. Heat tolerance and suppression of oxidative stress: Comparative analysis of 25 cultivars of the C_3 grass *Lolium perenne. Environmental and Experimental Botany* 78: 10–17.

SOLTIS, D. E., R. J. A. BUGGS, J. J. DOYLE, AND P. S. SOLTIS. 2010. What we still don't know about polyploidy. *Taxon* 59: 1387–1403.

SOLTIS, D. E., P. S. SOLTIS, AND J. A. TATE. 2004. Advances in the study of polyploidy since Plant speciation. *New Phytologist* 161: 173–191.

SOLTIS, P. S., AND D. E. SOLTIS. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences, USA* 97: 7051–7057.

SPECKMANN, G. J., J. P. JR, AND H. DIJKSTRA. 1965. The length of stomata as an indicator for polyploidy in rye-grasses. *Euphytica* 14: 225–230.

STEBBINS, G. L. 1947. Types of polyploids: Their classification and significance. *Advances in Genetics* 1: 403–429.

STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.

STEBBINS, G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London, UK.

STEBBINS, G. L., AND J. C. DAWE. 1987. Polyploidy and distribution in the European flora: A reappraisal. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 108: 343–354.

ŠTĚPÁNKOVÁ, J. 2001. Non-adaptive hypothesis of allopatric cytotype distribution in *Myosotis lamottiana* (Boraginaceae). *Folia Geobotanica* 36: 147–161.

STONE, G. N., S. NEE, AND J. FELSENSTEIN. 2011. Controlling for non-independence in comparative analysis of patterns across populations within species. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 366: 1410–1424.

STUTZ, H. C., AND S. C. SANDERSON. 1983. Evolutionary studies of *Atriplex*: chromosome races of *A. confertifolia* (Shadscale). *American Journal of Botany* 70: 1536.

TE BEEST, M., J. J. L. ROUX, D. M. RICHARDSON, A. K. BRYSTING, J. SUDA, M. KUBEŠOVÁ, AND P. PYŠEK. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109: 19–45.

THEODORIDIS, S., C. RANDIN, O. BROENNIMANN, T. PATSIOU, AND E. CONTI. 2013. Divergent and narrower climatic niches characterize polyploid species of European primroses in *Primula* sect. *Aleuritia. Journal of Biogeography* 40: 1278–1289.

TRABUCCO, A., AND R. ZOMER. 2010. Global soil water balance geospatial database. CGIAR Consortium for Spatial Information. Website http://www.cgiar-csi.org [accessed 30 January 2014].

TREIER, U. A., O. BROENNIMANN, S. NORMAND, A. GUISAN, U. SCHAFFNER, T. STEINGER, AND H. MÜLLER-SCHÄRER. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa. Ecology* 90: 1366–1377.

TYLER, B., M. BORRILL, AND K. CHORLTON. 1978. Studies in *Festuca*. X. Observations on germination and seedling cold tolerance in diploid *Festuca pratensis* and tetraploid *F. pratensis* var. *apennina* in relation to their altitudinal distribution. *Journal of Applied Ecology* 15: 219.

VAN LAERE, K., S. C. FRANÇA, H. VANSTEENKISTE, J. VAN HUYLENBROECK, K. STEPPE, AND M.-C. VAN LABEKE. 2011. Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii. Acta Physiologiae Plantarum* 33: 1149–1156.

VISSER, V., W. D. CLAYTON, D. A. SIMPSON, R. P. FRECKLETON, AND C. P. OSBORNE. 2014. Mechanisms driving an unusual latitudinal diversity gradient for grasses. *Global Ecology and Biogeography* 23: 61–75.

VOSHELL, S. M., R. M. BALDINI, R. KUMAR, N. TATALOVICH, AND K. W. HILU. 2011. Canary grasses (*Phalaris*, Poaceae): Molecular phylogenetics, polyploidy and floret evolution. *Taxon* 60: 1306–1316.

VOSHELL, S. M., AND K. W. HILU. 2014. Canary grasses (*Phalaris*, Poaceae): Biogeography, molecular dating and the role of floret structure in dispersal. *Molecular Ecology* 23: 212–224.

WAINES, J. 1994. High temperature stress in wild wheats and spring wheats. *Functional Plant Biology* 21: 705–715.

WARREN, D. L., R. E. GLOR, AND M. TURELLI. 2008. Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution; International Journal of Organic Evolution* 62: 2868–2883.

WIENS, J. J., D. D. ACKERLY, A. P. ALLEN, B. L. ANACKER, L. B. BUCKLEY, H. V. CORNELL, E. I. DAMSCHEN, ET AL. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* 13: 1310–1324.

WIT, F. 1958. Tetraploid Italian ryegrass (*Lolium multiflorum* Lam.). *Euphytica* 7: 47–58.

WOOD, T. E., N. TAKEBAYASHI, M. S. BARKER, I. MAYROSE, P. B. GREENSPOON, AND L. H. RIESEBERG. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences, USA* 106: 13875–13879.

YESSON, C., P. W. BREWER, T. SUTTON, N. CAITHNESS, J. S. PAHWA, M. BURGESS, W. A. GRAY, ET AL. 2007. How global is the global biodiversity information facility? *PLOS ONE* 2: e1124.